Agrobacterium-mediated transformation of maize

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Stable genetic transformation of maize is of vital importance to enable detailed functional analyses of genes involved in plant-microbe interaction. However, efficient maize transformation is still difficult to perform since only a few exotic genotypes are well amenable to this method and published protocols are only hardly reproducible in other labs. The goal of this work is to develop a reliable method of *Agrobacterium*-mediated gene transfer to highly embryogenic Hi II hybrid maize immature zygotic embryos in order to provide a powerful technical platform within the DFG-Forschergruppe 666. This consortium collectively aims to elucidate compatibility mechanisms of plants and fungal microbes. The induction of regenerable callus represents an essential step in the transformation process. Several media components were varied with the main focus on growth regulators. As a result, N6 basal medium supplemented with L-proline, casein hydrolysate and dicamba turned out to be most suitable for the efficient formation of somatic embryos at the callus surface. For the subsequent plant regeneration, a simple MS-based medium was utilised. Gene transfer was conducted using the *Agrobacterium tumefaciens* strain EHA105 harbouring a standard binary vector with the *bar* gene as selectable marker and the *gus*-intron reporter gene under the control of a doubled enhanced *CaMV35S* promoter. Several factors of the co-culture were examined to optimize transient GUS-expression of immature embryos. An elevation of acetosyringone concentration, the addition of the reducing agent dithiothreitol, the substitution of sucrose by maltose as osmoticum and carbohydrate source, and an increase of the *Agrobacterium* population density proved to be the most effective measures to improve the transformation protocol. First transformation experiments based on the newly developed N6-based medium resulted in 33 GUS expressing calli. The highest proportion of GUS-expressing calli (13%) was observed in an experiment using embryos of Hi II A x Hi II B with a size of 1.9 mm. So far, eight independent transgenic lines were confirmed by PCR. An analysis of gene integration patterns by Southern blot is under way.